UNUSUAL PARTITIONING BEHAVIOR OF CaATPase IN DIPALMITOYLPHOSPHATIDYLETHANOLAMINE/ DIELAIDOYLPHOSPHATIDYLCHOLINE MIXTURES

MARK JAWORSKY AND RICHARD MENDELSOHN
Department of Chemistry, Newark College of Arts and Sciences, Rutgers University, Newark, New Jersey 07102

ABSTRACT CaATPase from rabbit sarcoplasmic reticulum has been isolated, purified, stripped of its native lipids, and reconstituted into binary lipid mixtures of dielaidoylphosphatidylcholine (DEPC) and dipalmitoylphosphatidylethanolamine (DPPE) or acyl-chain perdeuterated DPPE (DPPE-d_{62}). The partitioning properties of the protein were determined from differential scanning calorimetry (DSC) and Fourier transform infrared (FT-IR) spectroscopy. Acyl-chain perdeuteration allows the separate determination of the order and melting characteristics of each lipid species with FT-IR. The binary lipid mixture has been shown to be phase separated in the gel state (Brauner, J. W., and R. Mendelsohn, 1986, *Biochim. Biophys. Acta*, 861:16–24). The solid phases present at low temperatures correspond to a pure DEPC phase and a mixed phase of DEPC/DPPE-d_{62}. Insertion of protein at 37°C leads to a domain of relatively protein-free DPPE-d_{62} and a phase containing both lipids plus CaATPase. We suggest that CaATPase selects a fixed composition (60% DEPC, 40% DPPE-d_{62}) for its immediate environment. The composition of the lipid in the immediate vicinity of protein is largely independent of the initial DEPC/DPPE-d_{62} ratios in the reconstitution protocol. The relevance of these results to observations of discrete domains in native membranes is discussed.

INTRODUCTION

Determination of the molecular basis of lipid–protein interaction in native and reconstituted biological membranes continues to be a topic of central importance in membrane biophysics (for an overview, see references 1–3). Toward this end, many physical studies have been carried out using purified integral membrane proteins reconstituted into a single phospholipid species, not infrequently, a disaturated phosphatidylcholine. Such simple lipids cannot mimic the possibilities for phase behavior in native membrane environments. In particular, according to the phase rule, lateral phase separation cannot occur over a finite range of temperatures in a one component lipid system. However, many recent studies (4–8) demonstrate the existence of persistent (perhaps phase-separated) domains in native membranes. Although the nature of these domains is unclear at present, it is reasonable to assume that the phase properties of the constituent lipids in the membranes play a role in domain formation, function, and maintenance.

To investigate mechanisms of lipid–protein interaction in environments that mimic those that may occur in vivo, we have, in previous studies from this laboratory (9–14), been involved with the reconstitution of glycophorin from the human erythrocyte membrane (9, 10) and CaATPase from rabbit skeletal muscle (11–14) into binary lipid mixtures. The latter are the simplest preparations that can mimic some of the in vivo possibilities and have been chosen in our studies to span a range of lipid classes and phase behaviors. The effects of protein on the miscibility properties of the lipid mixtures have been monitored with differential scanning calorimetry (DSC) and Fourier transform infrared (FT-IR) spectroscopy. The latter has been particularly useful in ternary systems (two lipids plus protein) in which one of the lipids has its acyl chains perdeuterated. The configuration-sensitive C-D stretching vibrations of the perdeuterated species can be monitored separately from and simultaneously with the C-H stretching vibrations of the other lipid component. Thus, the effect of protein on the order and melting characteristics of each lipid component may be determined.

Prior work from this laboratory has shown that CaATPase exhibited one of three types of partitioning preference in binary lipid systems, as follows: (*a*) No preferential association with either component in well-mixed lipid systems such as POPE/DDPC-d_{62} (14). (*b*) Selection of the lower melting component in lipid mixtures that showed partial or complete gel state phase separation (SOPC/DPPC-d_{62} [14] or DOPC/DPPC-d_{62} [12]). (*c*) The ability to induce gel state phase separation in a complex lipid

Offprint requests and other correspondence should be addressed to Dr. Mendelsohn.

Dr. Jaworsky's current address is Department of Chemistry, University of California, Riverside, CA 92521.

environment (i.e., DEPC/DMPC-d_{54}/native lipids mixture) (13).

In the current study, CaATPase has been reconstituted into a lipid mixture, 1,2 dielaidoylphosphatidylcholine (DEPC) and acyl-chain perdeuterated 1,2 dipalmitoylphosphatidylethanolamine (DPPE-d_{62}), that has been shown (15–17) to possess gel state phase separation and non-ideal mixing in the liquid crystalline phase. The effects of protein on lipid order and melting behavior have been monitored with DSC and FT-IR techniques. Unusual protein-induced alterations in lipid organization have been observed, consistent with substantial protein-mediated reorganization of domains in the bilayer.

EXPERIMENTAL

Materials

Isolation, Purification, and Reconstitution of CaATPase. CaATPase was isolated and purified from the rabbit skeletal muscle according to the procedure of MacLennan (18). Exchange of endogenous lipids was accomplished via the deoxycholate-mediated exchange protocol of Warren et al. (19), as later modified by Hidalgo et al. (20). The weight ratios (protein/deoxycholate/phospholipid) used in all samples were 1:1:2. Composition control was achieved in variation of the lipid/lipid mole ratios as indicated in Table I.

Samples were purified on discontinuous sucrose density gradients (15/30/50% for sample A; 30/40/50% for the others) with an overnight centrifugation at 150,000 *g*.

Vesicles were assayed for their chain length distribution by gas chromatography of their methylated acyl chains. The lipids were extracted according to the procedures of Bligh and Dyer (21), esterified with a BF_3-methanol solution, and extracted with diethyl ether. The methyl esters were analyzed on a 5890 A gas chromatograph (Hewlett-Packard Co., Palo Alto, CA) equipped with a methyl silicone gum capillary column by using a temperature program of 100°–183°C.

TABLE I
CHARACTERIZATION OF DEPC/DPPE-d_{62}/CaATPase COMPLEXES

Complex	Native SR	A	B	C	D
Total lipid protein mol ratio	30:1	256:1	130:1	52.1	87:1
ATPase activity (IU/mg protein)	1–6	0.74	0.65	0.91	0.5
Initial DEPC/DPPE-d_{62}*		50:50	67:33	67:33	81:19
Final DEPC/DPPE-d_{62}		24:76	36:64	45:55	67:33
Chainlength: No. of C = C					
12:0	0.9	—	—	—	—
14:0	0.9	1.2	2.5	—	—
16-h_{62}:0	30.4	3.1	3.5	5.4	0.8
16-D_{62}:0	—	68.0	55.9	43.5	29.2
18:0	8.2	—	3.0	8.2	3.1
18:1 *trans*	—	21.7	31.3	35.7	59.4
18:1 *cis*	22.6	3.7	3.8	7.2	7.6
18.2	34.7	2.3	—	—	—
18.3	2.4	—	—	—	—

*In reconstitution mixture.

ATPase activities were monitored with a coupled enzyme assay system (22). Lipid concentrations were determined via a lipid phosphorus assay (23), and protein concentrations were determined by the method of Lowry et al. (24).

Binary Lipid Mixtures. The binary lipid complexes for the reconstitutions were prepared by dissolving the appropriate mole ratios of lipids in $CHCl_3$, drying under a stream of N_2 gas, and evacuating in a dessicator (<1 torr) for 3–10 h to remove all traces of solvent. Rehydration was accomplished above the transition temperature of the mixture.

Methods

FT-IR. The samples were examined in a Harrick cell (6 or 12 μM path length) equipped with CaF_2 windows. Spectra were recorded with a Sirius 100 spectrometer (Mattson Instruments, Inc., Madison, WI) equipped with a mercury-cadmium-telluride detector. Routinely, 100 interferograms were collected, co-added, apodized with a triangular function, and Fourier transformed to give a resolution of 4 cm^{-1} with data encoded every 2 cm^{-1}. Temperature was controlled with a circulating bath (model A80; Haake Buchler Instruments, Inc., Saddle Brook, NJ) and monitored with a Bailey BAT 12 digital thermometer (Bailey Instruments, Inc., Saddle Brook, NJ) with a thermocouple sensor placed close to the windows of the cell but not in the optical path. Frequencies were measured with a center of gravity routine (25). The spectrum of water, matched for temperature and path length, was subtracted from all spectra. Residual sloping baselines were removed by a linear baseline leveling routine.

DSC. DSC experiments were performed in a Micro Cal, Inc. (Amherst, MA) MC1 unit. 0.7 ml of homogenate containing 3–5 mg of lipid was injected into the sample cell, and the same amount of buffer was used in the reference cell. Samples were heated at 24°C/h after an equilibration period of 1–2 h.

Supplies

Lipids were purchased from Avanti Polar Lipids, Inc. (Birmingham, AL). BF_3-methanol was purchased from Supelco, Inc. (Bellefonte, PA). Solvents were of the highest purity commercially available. Doubly distilled water was used in all buffers.

RESULTS

Binary Lipid Mixtures

The phase diagram for the DEPC/DPPE-d_{62} binary lipid mixture has been determined by DSC (17) and is shown in Fig. 1. The diagram depicts gel phase immiscibility from 0 to 70 mol% DPPE-d_{62}. Although there is substantial nonideality of mixing in the liquid crystal phase, a detailed examination of the liquidus line in the DPPE/DEPC system (16) indicates that there is no immiscibility in the liquid crystalline state, in contrast to a previous suggestion (15). To deduce the molecular nature of the phases present in the current mixture, the melting of each lipid component was monitored by FT-IR spectroscopy. Typical spectral data for the C-H and C-D stretching regions are shown in Fig. 2, *A* and *B*, respectively. For the DEPC component (Fig. 2 *A*), the asymmetric and symmetric CH_3 stretching modes appear near 2,956 and 2,872 cm^{-1}, respectively,

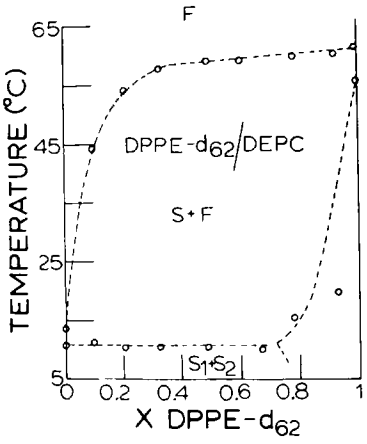

FIGURE 1 Phase diagrams for the DEPC/DPPE-d_{62} binary lipid mixture (see reference 15). The diagram was constructed from the set of onset and completion temperatures as obtained from the DSC data. Gel state immiscibility is evident from the horizontal solidus line. $S_1 + S_2$ indicates two distinct solid phases. S, solid; F, fluid.

whereas the antisymmetric and symmetric CH_2 stretching vibrations are observed near 2,920 and 2,850 cm^{-1} (26). In addition, there is a broad Fermi resonance band centered near 2,900 cm^{-1}, which appears as a shoulder (27). For the DPPE-d_{62} component (Fig. 2 *B*), the terminal CD_3 modes give rise to bands at 2,212 cm^{-1} (asymmetric stretch) and 2,169 cm^{-1} (symmetric stretch). The stronger bands near 2,194 and 2,089 cm^{-1} arise from antisymmetric and symmetric CD_2 stretching modes, respectively (28).

The melting of the DEPC component (Fig. 3) in a 67:33 DEPC/DPPE-d_{62} mixture is monitored by temperature-induced changes in the frequency of the CH_2 symmetric stretching band near 2,850 cm^{-1}. A sharp discontinuity appears between 11° and 13°C, close to the melting of the pure lipid (see inset of Fig. 3), and is hence thought to arise from a phase of nearly pure DEPC. This discontinuity is followed by a broad, noncooperative melting event that is not completed until ~43°C, hence is thought to arise from the melting of the DEPC component in a mixed phase of DEPC/DPPE-d_{62}. Evidence for this comes from the melting of the DPPE-d_{62} component (as monitored by temperature-induced changes in the frequency of the CD_2 symmetric stretching band near 2,100 cm^{-1}) of the binary lipid mixture (Fig. 3). A broad event is observed between 30° and 52°C, the beginning of which overlaps in temperature the broad melting of the DEPC component. The substantial decrease in the melting cooperativity and decrease in the transition temperature for the DPPE-d_{62} in the mixture compared with the pure component (T_m = 58°C; typical melting halfwidth, ~1°C) indicates that there is no pure DPPE-d_{62} phase in the mixture. The following summarizes the results from the FT-IR and DSC characterization (15) of this mixture: (*a*) There are gel phase domains of pure DEPC. (*b*) There are gel phase domains containing a mixture of DPPE-d_{62} and DEPC. There is no phase containing pure DPPE-d_{62}.

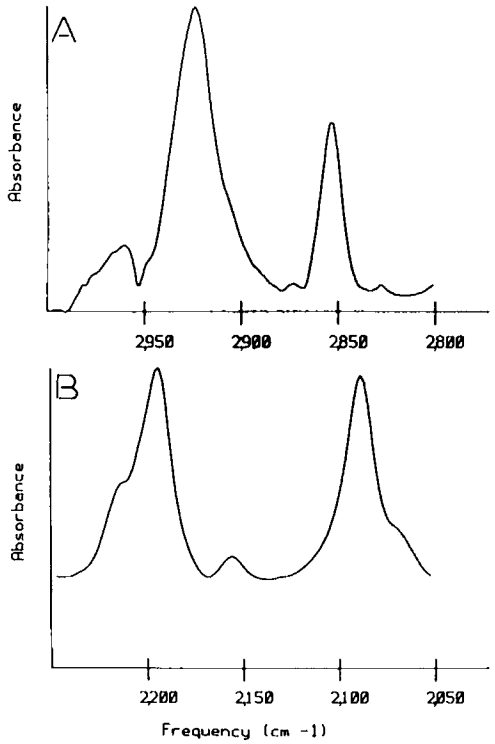

FIGURE 2 (*A*) Typical C—H stretching data for the DEPC component of a binary DEPC/DPPE-d_{62} mixture. The spectral region plotted is 2,800–3,000 cm^{-1}. Features are assigned in the text. (*B*) Typical C—D stretching data for the DPPE-d_{62} component of a binary DEPC/DPPE-d_{62} mixture. The spectral region plotted is 2,050–2,250 cm^{-1}. Features are assigned in the text.

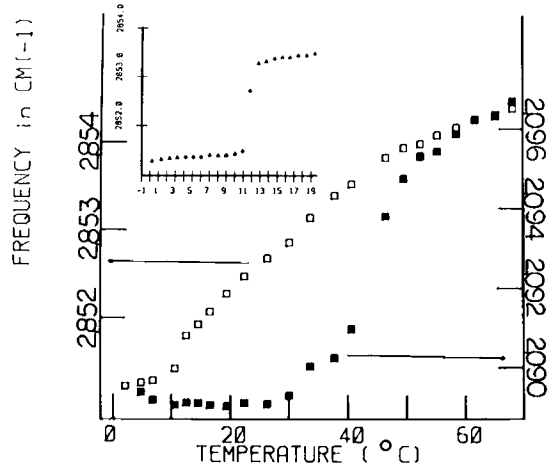

FIGURE 3 Melting of the components in a DEPC/DPPE-d_{62} 33:67 binary lipid mixture. The DEPC component is monitored by temperature-induced variation in the CH_2 symmetric stretching band (left-hand ordinate scale, points given by [□]). The DPPE-d_{62} component is monitored by temperature-induced variation in the CD_2 symmetric stretching band (right hand ordinate scale, points given by [■]). (*Inset*) Melting of pure DEPC (▲) at 11°–12°C. Same parameter as in the DEPC component of the mixture.

Ternary Complexes with CaATPase

Biochemical Characterization of Complexes. The results of the biochemical characterizations of ternary complexes are summarized in Table I. Four lipid–protein complexes of different overall lipid compositions were prepared and examined. The activities of the complexes are lower than have been achieved in other systems (12–14) using the same reconstitution protocols and enzyme preparations with similar starting activities. From the data in Table I, there does not appear to be a strong dependence of enzyme activity on the lipid level.

The fatty acid distribution of the lipid acyl chains reveals that the efficiency of reconstitution is high; 79–90% of the desired lipid species were successfully inserted into the reconstituted vesicles. We note that the number of undesired lipid molecules in samples B–D is substantially less than needed to sustain ATPase activity. In the least favorable circumstance at least one-half of the lipids in any putative boundary layer will consist of the desired species.

DSC Results. The thermotropic behavior of the four complexes was monitored by DSC and the endotherms are shown in Fig. 4. Included for comparison in Fig. 5 are DSC scans for four DEPC/DPPE-d_{62} binary lipid mixtures, which span the lipid composition range of the ternary systems. The starting, midpoint, and completion temperatures for the various transitions in both the sample and control systems are tabulated in Table II.

The similarity of the two main endotherms in each of the complexes A–C in Fig. 4 suggests that the compositions of the phases present do not vary greatly from one to the next. The primary effect of the relatively small variation in overall lipid composition is to change the relative proportion of each phase present, assuming that the areas under the transition can be used as an indicator of the extent of a particular phase. Comparison of the endotherms for lipid protein complexes A–C with those of binary lipid mixtures of the same approximate compositions (traces *A–C* in Fig. 5) show some startling differences. In the protein-free systems, the appearance of an endotherm at ~12°C (traces *B–D*) reveals the occurrence of domains of pure DEPC. The complexes (Fig. 4, traces *A–C*) show no such domains. In the protein-free systems, the FT-IR data (Fig. 3) show that the high temperature transitions are due to the melting of DEPC/DPPE-d_{62} domains. Mixing of the components thus results in an endotherm very much broadened from pure DPPE-d_{62}. The presence of protein, also, leads to a broadened endotherm at temperatures (maximum in heat capacity change at 48°–50°C) close to the high temperature trace in the control systems. In addition, however, an intense, reasonably cooperative event occurs with a midpoint of ~59°C (Table II), close to T_m for pure DPPE-d_{62}. It thus appears that the presence of protein

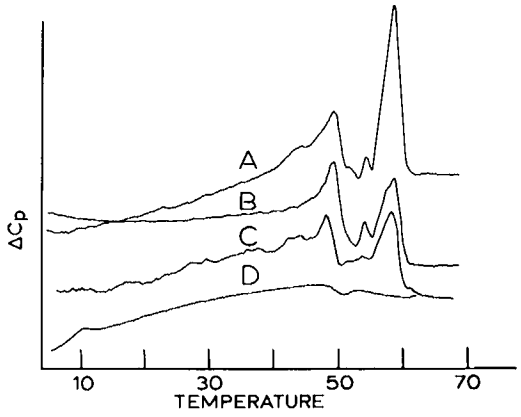

FIGURE 4 DSC traces for the first heating of DEPC/DPPE-d_{62}/Ca ATPase ternary complexes. *A–D* refer to the complexes of the same letter whose compositions are defined in Table I and whose transition temperatures are summarized in Table II. Significant noise in trace *C* is due to small amounts of material present. Note that the overall amplitudes of the *y*-axis are not comparable from one sample to the next due to differing amounts of material.

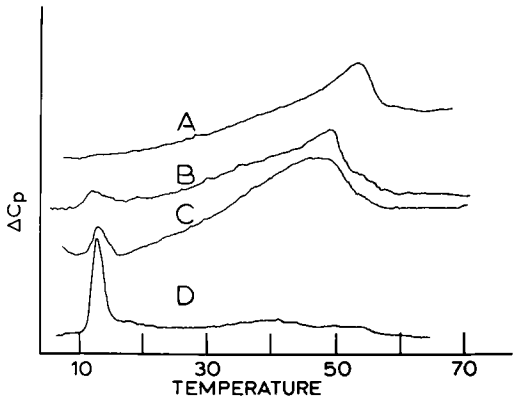

FIGURE 5 DSC traces for model systems, i.e., binary DEPC/DPPE-d_{62} mixtures of the following compositions (molar ratios): (*A*) 22:78; (*B*) 33:67; (*C*) 51:49; (*D*) 67:33.

TABLE II
TRANSITION TEMPERATURES FOR TERNARY COMPLEXES

Complex	Heating	T_s^{L*}	T_{max}^{L}	T_f^{L}	T_s^{M}	T_{max}^{M}	T_f^{M}	T_s^{H}	T_{max}^{H}	T_f^{H}
A	1	id	49.5	53.3	53.3	54.8	56.0	56.0	59.3	62.3
	2	id	54.3	57.0						
B	1	12	49.5	52.5	52.8	53.3	54.3	54.3	59.0	61.3
	2	9	53.5	57.8						
C	1	14	48.0	50.5				55.3	58.3	60.8
	2	id	53.4	57.8						
D	1	7	11	13	13	47	50			

id, ill-defined.

*Superscripts L, M, H, indicate the low, middle, and high temperature transitions, respectively. T_s, T_{max}, and T_f refer to the onset temperature, the temperature of maximum ΔC_p, and completion temperature, respectively.

results in the formation of domains of nearly pure DPPE-d_{62}. Finally, an additional weak endotherm is noted in traces *A* and *B* with a peak near 54°C. The visibility of this band in trace *C* is marginal.

To verify that the observed sharp transition near 59°C did not result from a separate DPPE-d_{62} vesicle population, three control experiments were carried out. First, the behavior of preformed DPPE-d_{62} vesicles in a sucrose gradient was monitored. It was observed that these appeared near the bottom of a 30/40/50% discontinuous gradient, i.e., at a much higher density than in any of the ternary complexes. Second, as discussed below, each ternary complex was subject to a second DSC heating in which the disappearance of the high-temperature endotherm was observed. Control experiments showed that distinct, preformed vesicle populations of DEPC and DPPE-d_{62} did not fuse under these conditions nor did separate populations of DPPE-d_{62} fuse with 50:50 mixtures of the two lipids. Thus, the disappearance of the high temperature peak in the protein-containing systems must have been due to a mixing of components in what was a homogeneous vesicle population. We note that the above argument assumes that neither native nor denatured CaATPase mediates fusion in this system. Finally, one experimental preparation of a ternary complex resulted in two bands in the sucrose gradient. Both bands contained two lipids plus protein, albeit at different molar ratios. Both bands showed the sharp endotherm at 59°C during the first DSC heating. Such behavior is inconsistent with a separate population of DPPE-d_{62} in the preparation, since the latter would float at its own density in the gradient.

The DSC results for a second heating of complexes A–C are shown in Fig. 6. The two or three transitions observed in the first heating of each complex disappear and are replaced by a single broad endotherm. The DSC traces are similar in each instance and, except for the absence of a DEPC peak near 11°C, resemble the data for the binary lipid controls (see Fig. 5) of similar overall composition.

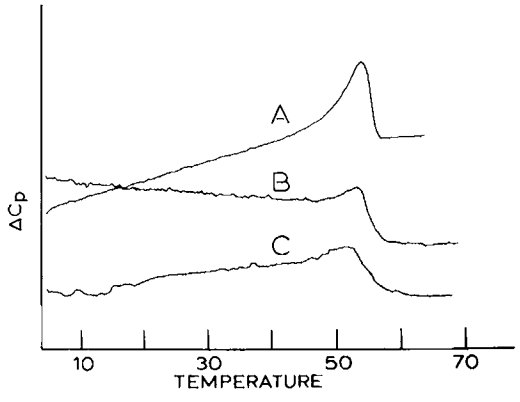

FIGURE 6 DSC traces for the second heating of DEPC/DPPE-d_{62}/CaATPase ternary complexes. The letters refer to the particular complex. Further repeats of the heating produced no further changes.

FT-IR Results

Lipid Phase Behavior in Ternary Systems. FT-IR data for the thermotropic behavior of the DEPC and DPPE-d_{62} components of ternary complex A upon first and second heating are shown in Fig. 7, *A* and *B*, respectively. The parameters plotted for the DEPC and DPPE-d_{62} components are the same as for the protein-free system depicted in Fig. 3. Data for complexes B and C (see Table I for composition data) are largely similar and so are not shown. The DEPC component (Fig. 7 *A*) upon first heating undergoes a broad melting event from ~20° to 46°C, in fair accord with the low temperature DSC endotherm. Upon second heating, the completion temperature is increased to ~55°C.

The DPPE-d_{62} (Fig. 7 *B*) shows three discrete melting regimes upon first heating. An initial, weak noncooperative

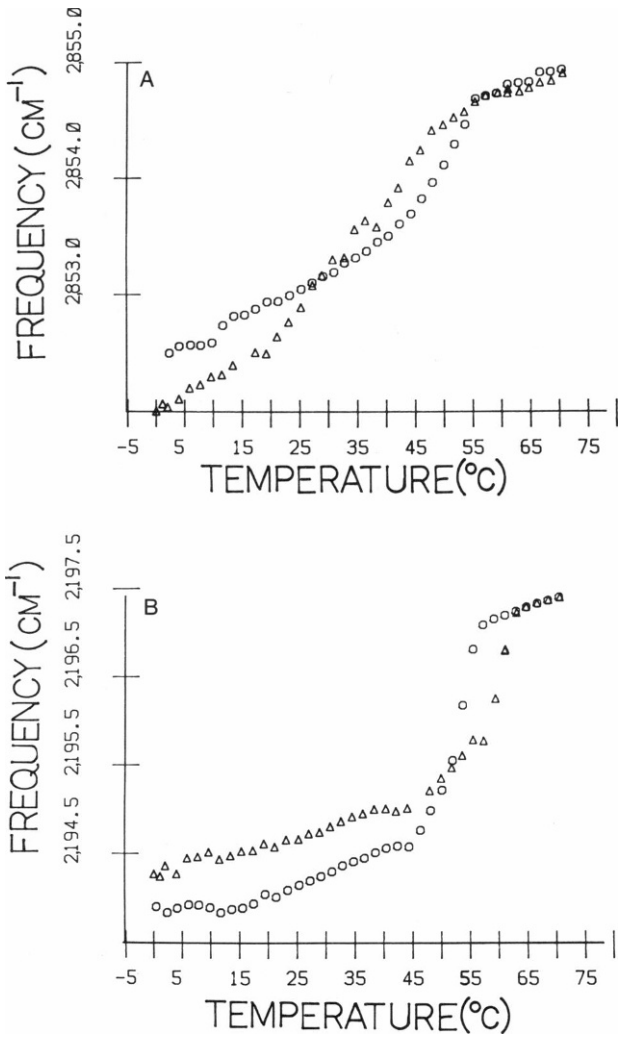

FIGURE 7 (*A*) FT-IR melting profiles for the DEPC component of complex A during a first heating (Δ) and a second heating (O). The parameter plotted is as in Fig. 3. (*B*) FT-IR melting profiles for the DPPE-d_{62} component of complex *B* during a first heating (Δ) and a second heating (O). The parameter plotted is as in Fig. 3.

event between 10° and 45°C is followed by a second more cooperative endotherm between 45° and 55°C and a sharp transition between 57° and 63°C. The latter two transition ranges correspond to the two high temperature DSC events (Fig. 4 *A*). The conclusion from these data is that the first DSC endotherm reflects the melting of DEPC with a small amount of DPPE-d_{62}, while the final two transitions reflect melting events of domains of relatively pure DPPE-d_{62}. The second heating of the complex results in the merging of the two high temperature transitions and a resultant event between 45° and 55°C, overlapped with the lower noncooperative transition.

Protein Secondary Structure. The spectral region containing the amide I (peptide bond C═O stretch) and the amide II (a mixture of peptide bond C—N stretch and N—H in-plane deformation) is shown for three temperatures in Fig. 8. The spectra have been subject to slight resolution enhancement (29), but no subtraction of the liquid water absorption was attempted.

At 36.8°C, the amide I region reveals (Fig. 8) peaks near 1,625 and 1,685 cm^{-1} as well as a feature near 1,660 cm^{-1}. It is probable that the positions of maximum intensity reflect slight shifts from the true peak values because of underlying interference from liquid water absorption near 1,640 cm^{-1}. The pair of features at 1,625 and 1,680 cm^{-1} are assigned to β-antiparallel sheet secondary structure (reference 30 for assignments), whereas the band at 1,660 cm^{-1} arises from α-helical amide I modes. At 52.3°C, above the thermal denaturation of the protein which occurs at 45°–50°C (29), the β-sheet bands seem to be enhanced in intensity at the expense of the α-helical band. This result, although difficult to ascertain with certainty from Fig. 8 due to the presence of H_2O, is consistent with earlier results (29) for native SR in D_2O suspension.

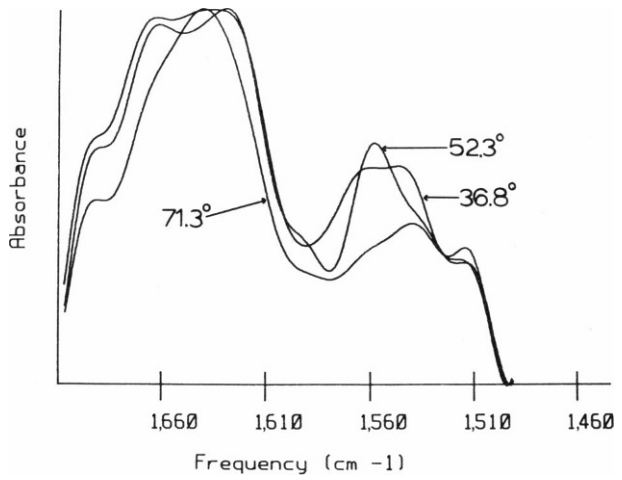

FIGURE 8 The amide I (1,620–1,680 cm^{-1}) and amide II (1,520–1,570 cm^{-1}) vibrations for CaATPase at three temperatures. The peak due to H_2O bending at ~1,640 cm^{-1} has not been subtracted. Spectral resolution has been slightly enhanced.

At 71.3°C it is evident that an additional process has taken place. The β-sheet bands are diminished in intensity and are replaced by a new spectral feature near 1,650 cm^{-1} assigned to irregular (random coil) secondary structure. Concomitant changes appear in the amide II spectral region (1,520–1,570 cm^{-1}).

DISCUSSION

A substantial rearrangement of the constituents of the separated gel phases evidently occurs upon insertion of CaATPase into the DPPE/DEPC system. In the binary lipid mixture, the phases present at low temperature consistent of a nearly pure DEPC phase and mixed DEPC/DPPE-d_{62} phase. There is no pure DPPE phase present. In contrast, insertion of CaATPase results in a system containing a mixed DEPC/DPPE phase along with a phase whose DSC characteristics (Fig. 4) and FT-IR melting profiles (Fig. 7) suggest it to be nearly pure DPPE-d_{62}. An initial explanation would be that DPPE-d_{62} is excluded from the vicinity of protein. This type of description, i.e., exclusion of the higher melting component, has been invoked for phase-separated lipid systems reconstituted with CaATPase (12). However, for the following reasons, the explanation is incomplete in the current system: (*a*) When CaATPase interacts with pure DEPC, the melting of the lipid is considerably reduced in temperature from the pure lipid (13). In the current case, the DEPC component of the ternary complexes melts at a higher temperature than DEPC in the binary controls. An additional lipid component in the vicinity of protein is thus implicated. (*b*) There is FT-IR evidence for some DPPE-d_{62} melting between 10° and 45°C, where the first broad DSC endotherm is noted.

It is tentatively suggested that a fraction of the DPPE-d_{62} component is selected by the protein for its immediate environment along with the DEPC. Furthermore, this fraction of DPPE is relatively independent of the lipid compositions used in the reconstitution protocol, as judged by the similarity of the first DSC endotherm in each of complexes A–C. Comparison of this endotherm with the DSC traces of binary lipid mixtures indicates that the lipid composition of the protein-containing phase is approximately 60:40 DEPC/DPPE-d_{62}.

To test this description, CaATPase was reconstituted into complex D containing a 66:34 mole ratio of DEPC/DPPE. If the current description is correct, there ought to be no discrete DSC endotherm from a DPPE-rich phase, as all the DPPE is supposedly tied up with protein and can no longer melt cooperatively. The DSC results, shown in Fig. 4 *D*, in fact reveal no endotherm attributable to a pure DPPE phase. A broad transition is noted from ~15° to 51°C as in samples A–C. No higher temperature endotherms are seen. Thus, this particular lipid composition is close to that required for the immediate environment of protein. In fact, a small residual transition near 11°C,

possibly arising from a relatively pure DEPC phase, is observed. The experiment thus provides further evidence for a particular lipid composition being required by protein. In complex D, the excess DEPC is excluded from the protein surface.

In addition to information about lipid environments of CaATPase, the current results suggest a dependence on the lipid environment of the protein secondary structures achieved upon thermal denaturation. CaATPase in native SR (29) undergoes denaturation from a predominantly α-helical to a predominantly β-sheet form at 45°–50°C, with no indication of additional major alterations in secondary structure as the temperature is raised. In contrast, the current study shows a second transition, from β-sheet to random coil, at temperatures that correspond to the melting of lipid domains enriched in DPPE-d_{62}.

The interaction of denatured (previously heated) protein (Fig. 6) with lipid is greatly different from that of the native CaATPase. No gel phase domains corresponding to either lipid component are observed. The DSC endotherms are similar to the binary lipid mixtures of the same compositions. It is possible that the thermally denatured CaATPase is highly aggregated and forms a separate phase, possibly at the surface of the lipid. Under these conditions, lipid organization would resemble that in the protein free systems (compare Fig. 6, *A–C* with Fig. 5, *A–C*).

Finally, we address the possible in vivo implications of this study. There have been many observations of "domain" formation in the membranes of intact cells (4–8). These are usually identified by techniques such as the persistence of fluorescence from localized areas. The molecular origin of domains cannot be elucidated from fluorescence measurements. FT-IR studies of model systems can serve to enumerate the possibilities that may occur in vivo. The advantage of the method, while it lacks the sensitivity of fluorescence, is that it allows identification of the domains and their assignment to a particular species. The combination of DSC and FT-IR in the current work reveals a large protein-induced reorganization of lipid gel phase domains. Extension of FT-IR methods to multicomponent systems in vitro is straightforward as long as the lipid species contains a distinguishing spectroscopic feature, such as the C-D stretching modes of perdeuterated acyl chains. In vivo studies depend upon the ability of the investigator to insert such species into the organism under study. The feasibility of such an approach has been demonstrated (30).

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